Stearn

The Mechanism of Swelling in Protein Colloids

THE MECHANISM OF SWELLING IN PROTEIN COLLOIDS

BY

ALLEN EDWIN STEARN

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.....191

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Allen Edwin Stearn

ENTITLED The Mechanism of Swelling in Protein Colloids.

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE OF.....

Richard C. Tolman
In Charge of Thesis

W. A. Noyes
Head of Department

Recommendation concurred in:*

Committee on Final Examination*

*Required for doctor's degree but not for master's.

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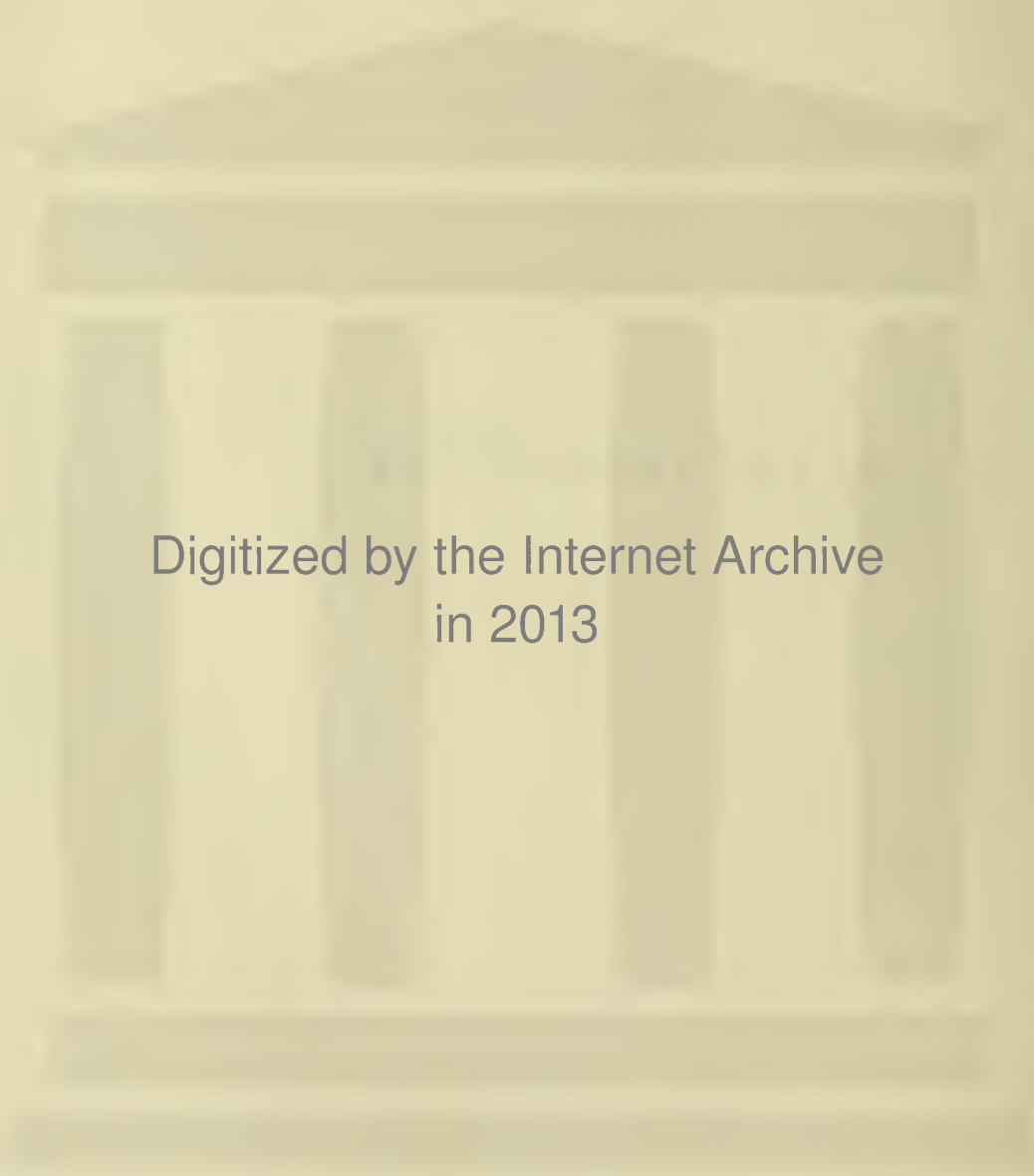
I. INTRODUCTION.



The swelling of colloidal gels in an acid or alkaline solution is a familiar phenomenon, as is also the effect of salts on such a system not only in retarding or inhibiting the swelling but in causing a gel already swollen to shrink almost to its normal size. Fischer (Bibl. A.I) has done the most comprehensive work in this field, confining himself, however, to the hydrophylic colloids as being closely related to the body tissues though the phenomenon is not so confined.

He has studied the effect of various acids and alkalis on the swelling of proteins and the degree of inhibition brought about by various electrolytes. From this data he could arrange the common anions in the order of their effectiveness in knocking down swelling by comparing the effect of a series of salts with a common cation; and by the same procedure was able to arrange such a series of cations. The effect was found to be additive and thus one might predict the most effective combinations. These in general were found clinically to be the most effective salts for dehydrating body tissues and are the common cathartics and diuretics.

However, in none of this work was there any attempt to explain in any way the mechanism of the swelling. A few theories have been advanced. H. R. Procter (Bibl. B.III) has put forth the theory that when dry gelatin comes to equilibrium in a solution of Hydrochloric acid, gelatin, acting as a di-acid base forms a di-hydrochloride, analagous to such substances as Analine hydrochloride probably, which he calls gelatin hydro-

chloride. This complex salt, he assumes, forms a molecular solution which at low concentrations is completely ionized and the swelling is due, then, to osmotic pressure.

"The theory assumes that the jelly is a molecular network in which the water, the acid, and the protein are within the sphere of each other's molecular attractions, and therefore homogeneous in the same sense as any other solution." This theory he supports by the fact that this swelling which reaches a maximum at a very low concentration of the external acid subsequently diminishes in a hyperbolic curve as the concentration of the acid is further increased.

From this fact coupled with the fact that the neutral salt of Hydrochloric acid, namely sodium chloride will decrease the swelling almost to complete dehydration he concludes that the contraction is "obviously" due to the anion of the acid. One very significant fact is not taken into account here at all, namely that sodium chloride is not the only salt effective in knocking down the swelling, nor is it even the most effective. From Fischer's work, a good deal of which has been confirmed in this laboratory during the year, the salts most effective in dehydration are magnesium citrate, Tartrate, etc. The chlorides of sodium or potassium being, as a matter of fact, among the least effect of the common salts.

There are two points which may be argued against osmosis as a complete explanation as offered by Procter. If the jelly is to be thought of as a molecular solution we have no semipermiable membrane. Then if osmotic phenomena explained completely the mechanism of the swelling we should not be able

to knock down the swelling unless we added a salt which contained the anion in common with the acid. This, of course, we know is not true and we must look to some property common to all salts for an explanation.

Moreover gelatin in its behavior toward alkalis shows all of these phenomena even, as Fischer has found, to a more marked degree than in its behavior toward acids. It is amphoteric and might just as well be called a di-basic acid as a di-acid base.

So far as the actual data is concerned, Procter's curve for the amounts of acid added to the gelatin with the change in concentration of the external acid was obtained by Herzog and Adler (Bibl. B. V) who used powdered skin in place of gelatin. We have been able to obtain the same curve with both gelatin and blood fibrin using various mineral acids but find it does not seem to hold for organic acids. Freundlich (Bibl. A. II) looks upon this adding of acid to the colloid as a surface adsorption phenomenon analogous to the adsorption of gases by charcoal. By plotting in logarithmic form their data Herzog and Adler obtained straight lines, or approximately straight lines when they used solutions of strong acids. For weak acids such as acetic, oxalic, etc. they obtained a curve which, if the actual amounts adsorbed and actual concentrations had been plotted instead of their logarithms, would have approached a straight line.

Indeed, if instead of logarithms, they should plot the actual data for behavior of strong acids they would obtain a curve of the same shape as Procter's "gelatin chloride" curve,

which shows that up to a certain low concentration the amount of acid taken up by the colloid rises very rapidly with increasing concentration. From this point the acid adsorbed increases only extremely slowly even for large ranges of concentrations. These curves, as will be shown later, agree with the results obtained in this laboratory during the year from work on both gelatin and blood fibrin, using various acids both strong and weak.

Gelatin and blood fibrin were chosen for obtaining the data submitted in this paper since they are two representative proteins which can be fairly readily obtained in a fairly pure state. Besides being very important substances themselves, they have been used by most investigators along this line and results on them can be more easily compared with those of others.

Fischer (Bibl. A I) has pointed out the physiological significance of the effect of acids, alkalis and salts on protein colloids and the work whose results are here presented was undertaken to obtain some data which might be general enough so that some light might be thrown on the mechanism of protein swelling, in view of the fact that osmotic phenomena do not seem to give the complete story.

II. EXPERIMENTAL METHODS.

A. MATERIALS

The same sample of blood fibrin was used throughout the year. It was a sample of Kahlbaum preparation which was purified before used by the following treatment. It was first ground to a coarse powder and then repeatedly washed with tenth normal hydrochloric acid. It was then washed repeatedly with distilled water to remove the acid. When the acid was practically removed - less than one ten thousandth normal - the water was carefully drained off, and the fibrin was dried in a current of warmed air for several days. In this way the remainder of the acid was completely volatilized.

The gelatin used was "blue label" gelatin, the purest obtainable. The same sample was used throughout.

The salts used were either Baker or Kahlbaum preparations; and with the exception of a few cases unopened bottles were obtained.

B. LINES OF ATTACK.

I. SWELLINGS.

In general two methods of procedure have been used. During the early part of the year a good deal of work was on the swelling of fibrin and gelatin in acids and alkalis and an attempt was made to study the specific effect of various types of ions in reducing the swelling, for instance whether a di-valent anion was more effective than a univalent one, whether or not the cation was the effective agent in reducing swelling in alkalis and the anion in acids or not.

For this work the powdered fibrin lent itself very

well. Here we could weigh or measure out a small amount into a tube of uniform cross section and mix it thoroughly with the salt and acid mixture. By measuring the heights we could get a measure of the amount of swelling. The gelatin as it comes in the package is not in a convenient form to use. To get it into a convenient form it was dissolved in a very little hot water, and poured into a flat bottomed dish to the depth of a few millimeters. When it cooled enough to be stiff it was cut into small squares, placed on a glass plate and dried thoroughly in a current of air.

The method of determining the swelling of the gelatin slabs was slightly different than for the fibrin. The slabs of dry gelatin were weighed, placed in small dishes and covered with the solution. When the swelling was to be determined they were removed, dried quickly between filter paper and weighed.

A. APPARATUS

For the work on the fibrin some special tubes were obtained. These were made from tubing one half inch in diameter and were seven inches long. They were made with flat bottoms in order to make the measurement of the height of the column of fibrin a correct measure of its swelling. These tubes do away with two sources of error which it seems to me must enter all such work which has been done using the common test tubes. The curvature of the bottom may not be the same, and even in test tubes supposed to be the same size their internal diameter may be different if not made from the same piece of tubing. Then, of course, with a round bottomed tube the height of the fibrin column is by no means a measure of its swelling.

For gelatin we found it convenient to use small, plain

glass finger bowls about four and a quarter inches in diameter and two and a quarter inches in depth.

B. SOLUTIONS.

The acid used for the work was, in general, hydrochloric. This was standardized against a standard solution of sodium hydroxide which was standardized with oxalic acid. Sodium hydroxide was used for the alkali. These solutions were normal and were diluted up to the required concentration when used.

A series of double normal salt solutions was made up. This consisted of three uni-univalent salts, the chlorides of Lithium, sodium and potassium; three uni-bivalent salts with a bivalent anion, the sulfates of Lithium, sodium and potassium; (the solubility of potassium sulfate necessitated a normal solution instead of a double normal); and three uni-bivalent salts with bivalent cations, the chlorides of Magnesium, Calcium and Barium. By weighing the proper amounts of salts to within half a gram and making them up to two liters, solutions were obtained which were within a per cent of being double normal. This is amply accurate as was shown from the slight differences in effect due to slight differences in salt concentration.

II. ADSORPTION.

The latter part of the year was spent for the most part in determining the curves for the adsorption of various acids by gelatin and blood fibrin, and of the analogous behavior of salts and its effect on the action of the acids.

For this purpose the aforementioned finger bowls were used. A weighed sample of gelatin or fibrin was placed in one of them and a known volume of solution was added - usually 100^{cc}

to two grams - the system was let stand twenty four hours and the concentrations of the acid, or salt, or salt and acid if it was a mixture was determined in both the original solution and the liquid in the dish.

The acid was titrated with a solution of sodium hydroxide of suitable normality using phenolphthalein as an indicator, the alkali being standardized against oxalic acid. The salt was determined by Volhard's Method. Ferric nitrate was used as an indicator. In the case of the mixture of sodium chloride and hydrochloric acid the total chloride was of course determined, the acid titrated, and the salt determined by difference but since in most cases the amount of salt was much larger than acid - the acid being kept at one hundredth normal - no large error would result from this. Swellings were also run on all these systems by making up tubes which contained the same proportions of colloid and solution.

III. MICROSCOPIC.

A little work was undertaken with a microscope to see whether or not some light could be obtained as to the structure of the gelatin jelly. With methylene blue and cochineal were tried. It was difficult to interpret anything seen, though we shall speak more of this later.

III. EXPERIMENTAL RESULTS.

A. SWELLINGS:

A large volume of data was obtained along this line. The results, in general, confirmed those obtained by Fischer. We were able to get swellings in hundredth normal HCl with gelatin up to sixty times its original weight.

Table I shows the swelling of Fibrin in equinormal solutions of various salts. Table II is the same for gelatin. Both represent the state of affairs after about 110 hours.

TABLE I - Fibrin

	Medium	Ref. to swelling in H_2O	Height in m. m.
1	H_2O	1	27
2	N LiCl	1.19	32
3	N NaCl	1.22	33
4	N KCl	1.22	33
5	N Li_2SO_4	1.26	34
6	N Na_2SO_4	1.11	30
7	N K_2SO_4	1.04	28
8	N $MgCl_2$	1.40	38
9	N $CaCL_2$	1.59	43
10	N $BaCl_2$	1.30	35

TABLE II - Gelatin

Medium	Max. swelling in % of original wt.	Max. swelling Ref. to swelling in H_2O
H_2O	1185%	1
NLiCl	2120%	1.79
NNaCl	2260%	1.9
NKCl	1920	1.62
NLi_2SO_4	1275	1.08
NNa_2SO_4	1220	1.03
NK_2SO_4	1470	1.24
N$MgCl_2$	2140	1.81
N$CaCl_2$	1835	1.55
N$BaCl_2$	Dissolved	

TABLE III

	Medium	FIBRIN Swelling in M.M.	FIBRIN Swelling Ref. to H2O control	FIBRIN Swelling in % of original	GELATIN Swelling referred to H2O control	Remarks
1	H_2O	29	1	1730%	1	Quite firm
2	$\frac{n}{50}$ HCl	92	3.17	3065	1.77	" "
3	$\frac{n}{50}$ NaOH	91	3.14	6140	3.54	Firm but large
4	$\frac{n}{50}$ HCl&N LiCl	25	.81	1215	.70	Soft
5	$\frac{n}{50}$ HCl&N NaCl	26	.90	655	.38	Firm
6	$\frac{n}{50}$ HCL&N KCl	25	.81	960	.55	"
7	$\frac{n}{50}$ HCl&N Li_2SO_4	28	.965	1060	.61	Tough
8	$\frac{n}{50}$ HCl&N Na_2SO_4	25	.81	700	.40	"
9	$\frac{n}{50}$ HCl&N K_2SO_4	28	.965	895	.52	"
10	$\frac{n}{50}$ HCl&N $MgCl_2$	31	1.07	1075	.62	Soft
11	$\frac{n}{50}$ HCl&N $CaCl_2$	32	1.10	1030	.60	Very soft
12	$\frac{n}{50}$ HCl&N $BaCl_2$	33	1.14	Dissolved		
13	$\frac{n}{50}$ NaOH&N LiCl	38	1.31	1810	1.05	Firm
14	$\frac{n}{50}$ NaOH&N NaCl	39	1.345	1870	1.08	"
15	$\frac{n}{50}$ NaOH&N KCl	40	1.38	2300	1.33	"
16	$\frac{n}{50}$ NaOH&N Li_2SO_4	35	1.21	1520	.88	Tough
17	$\frac{n}{50}$ NaOH&N Na_2SO_4	33	1.14	1265	.73	"
18	$\frac{n}{50}$ NaOH&N K_2SO_4	37	1.27	1115	.65	"
19	$\frac{n}{50}$ NaOH&N $MgCl_2$	41	1.41	1975	1.14	Sticky
20	$\frac{n}{50}$ NaOH&N $CaCl_2$	42	1.45	2275	1.31	Very soft
21	$\frac{n}{50}$ NaOH&N $BaCl_2$	41	1.41	Dissolved		

NOTE: $\frac{n}{100}$ acid and alkali used with gelatin instead of $\frac{n}{50}$

Table III is a representative collection of data from an experiment chosen from a number of the same kind. It shows the effect of various salts on swollen fibrin and gelatin.

Upon first sight it might appear that we were getting as great swellings from certain salts as from acids but it must be borne in mind that the acids are very dilute while the salts are normal. Indeed the effect on Fibrin is indistinguishable whether normal or double normal NaCl be used while $\frac{n}{100}$ salts have practically no effect on either fibrin or gelatin.

Several other things must be taken into account the most important of which is the specific effect of various ions. For instance the Barium ion seems to be especially vigorous in tearing the gelatin slab to pieces, any soluble salt of Barium being effective so far as we could tell. This agrees, of course, with the fact so well known clinically that Barium salts are very active poisons.

LiCl had more or less an analagous effect though to a much slighter degree. This was not due to the Lithium ion as the sulfate did not have the property of tearing the gelatin to pieces.

As will be noted from the "remarks" in Table III the slabs were not all in the same condition. Some were "tough" and could be easily dried and weighed. Others were soft. Still others were firm but stuck to the filter paper. From this two difficulties arose. Many times on weighing was all that a slab would stand and there was no way of telling whether or not it had reached a maximum except by letting it stand as long as previous experience taught us. Also on account of the different degrees of firmness it was very difficult to satisfy ourselves

that all were equally well dried before weighing.

As might be expected the data is not as clear cut as one would desire, and another method of attack was started. It might be pointed out here that the salts which in themselves produced the least swelling seem most effective in general in knocking it down.

B. ADSORPTION.

In the following tables "C" represents the normality concentration of the solution before the swelling took place and C' the concentration afterward. M is the number of mols of acid adsorbed per gram. The dilute solutions were titrated with $\frac{N}{100}$ or $\frac{N}{50}$ NaOH and the more concentrated ones with $\frac{N}{10}$NaOH. The number of mols adsorbed was calculated from the formual $M = V\frac{(C-C')}{\text{wt sample}}$. In the curves C is plotted against M and also against the swellings, which were measured for all solutions used. "V" in every case = 100 c.c.

TABLE IV.

GELATIN & HCl

Wt. Sample	C	C'	M	Swelling
3.359 grm	.885	.855	.00087	
3.612	.885	.854	.00086	
4.713	.442	.416	.00056	
3.595	.3265	.305	.00060	
4.493	.2177	.191	.00059	
2.643	.1078	.095	.00048	14.2 Times
3.047	.089	.0734	.00051	
3.300	.089	.0725	.00049	15.5 ")maximum somewhere
2.681	.055	.042	.00048	)in here
3.038	.018	.006	.000395	13.6 "
3.146	.018	.0058	.00039	
3.646	.0103	.00255	.00021	7.3 "
3.192	.0103	.0038	.000205	
2.824	.0051	.0015	.000165	6.4 "
3.162	.0051	.0016	.00011	

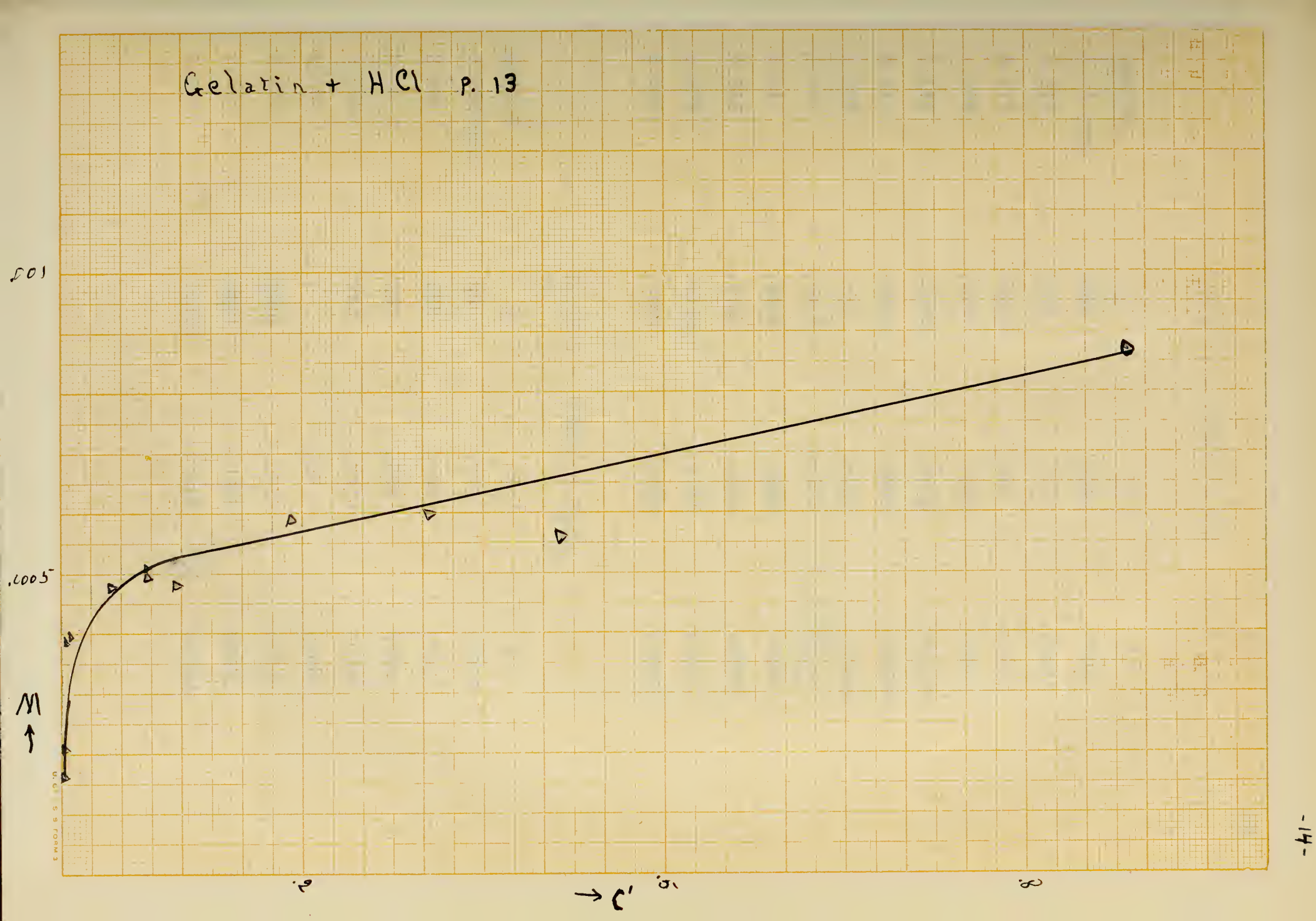
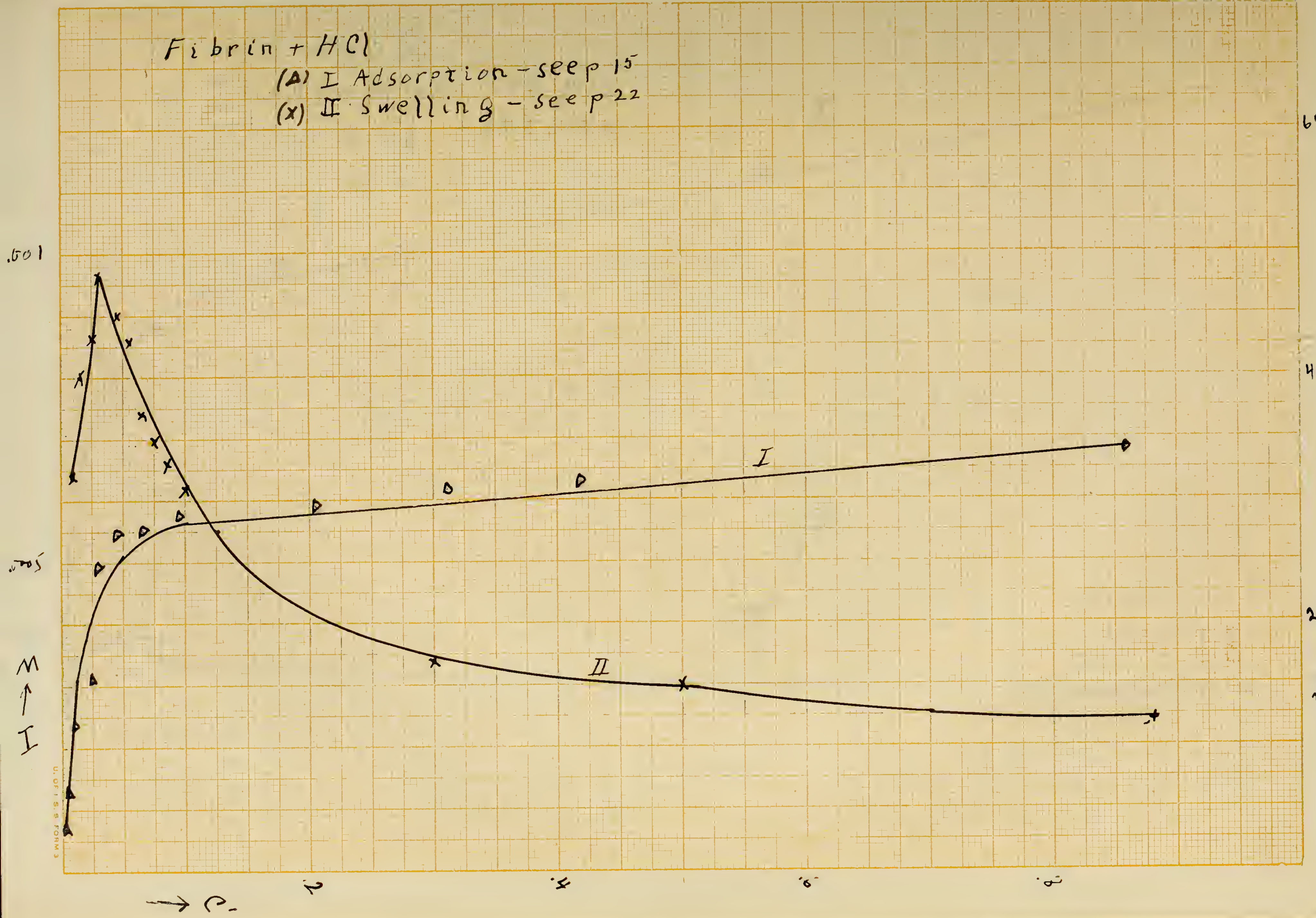

Gelatin + HCl p. 13
.001
.0005
M
λ'

TABLE IV - FIBRIN & HCl

SAMPLE	C	C'	M
3.95 grm	.885	.858	.00068
3.673	.442	.419	.00063
2.177	.3265	.313	.00062
2.277	.2177	.2043	.00059
2.177	.1078	.0953	.000575
3.96	.0885	.0665	.000555
2.087	.055	.0435	.000550
2.20	.0408	.0300	.00049
2.21	.0309	.0240	.00031
3.845	.018	.0090	.000235
3.994	.0103	.0053	.00013
3.947	.0051	.0023	.00007

TABLE V - FIBRIN & H_2SO_4

SAMPLE	C	C'	M
2.256 grm	.96	.931	.00128
2.031	.592	.569	.00113
2.18	.305	.283	.00101
2.272	.204	.1815	.00099
2.269	.1	.08	.00088
2.270	.0504	.0312	.00085
2.269	.0201	.0086	.00051
2.257	.0101	.0039	.000275



Fibrin + HCl
(Δ) I Adsorption - see p 15
(x) II Swelling - see p 22
.001
.0005
I
II
60
40
20
Ht in mm.
↑ II
M ↑ I
.2
.4
.6
.8
→ C
U. OF I. S. S. FORM 3

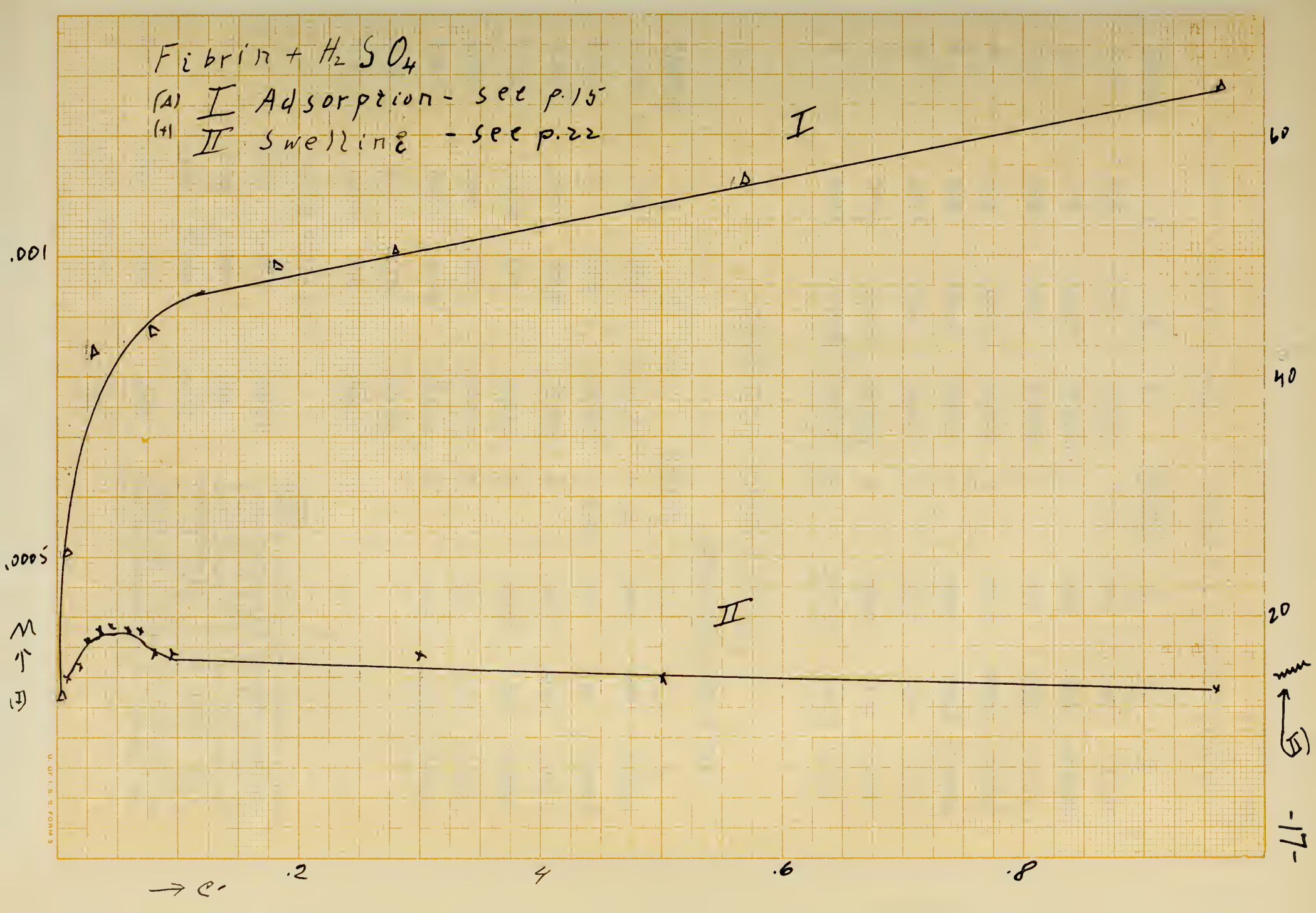

Fibrin + H_2SO_4
(A) I Adsorption - see p. 15
(+) II Swelling - see p. 22
I
II
.001
.0005
60
40
20
.2
4
.6
.8
→ c-

TABLE VI - FIBRIN & HNO_3

SAMPLE	C	C'	M
2 Gm	1.008	.991	.00085
2	.499	.4825	.000825
2	.305	.288	.00085
2	.201	.1847	.00081
2	.103	.0867	.00081
2	.0512	.0355	.00079
2	.0206	.01235	.00042
2	.0104	.00505	.00027

TABLE VII - FIBRIN &HCOOH

SAMPLE	C	C'	M
2 Gm	1.048	1.040	.00040
2	.523	.518	.00025
2	.305	.3015	.00017
2	.2045	.2020	.00012
2	.1026	.1006	.00010
2	.0515	.0495	.00010
2	.0204	.0184	.00010
2	.00995	.00815	.0009

TABLE VIII - FIBRIN & $HC_2H_3O_2$

(Duplicate Run 5 Hrs.)

SAMPLE	C	C'	M
2.157 Gm	.903	.888	.00070
2.21	.61	.598	.00055
2.20	.307	.3007	.00029
2.20	.2033	.199	.00020
2.20	.104	.100	.00018
2.20	.0515	.0483	.00015
2.20	.021	.0192	.00008
2.20	.0105	.009	.00007

SAMPLE	C	C'	M
2 Gm	.903	.895	.00040
2.125	.61	.604	.00028
2	.307	.3038	.00016
2	.2033	.2005	.00014
2	.021	.0196	.00007
2	.0105	.00913	.00007

TABLE VIII-A

2	2.067	2.028	.00195
2	3.068	2.988	.004
2	5.130	5.025	.0052
2	7.740	7.650	.0045
2	10.320	10.200	.006

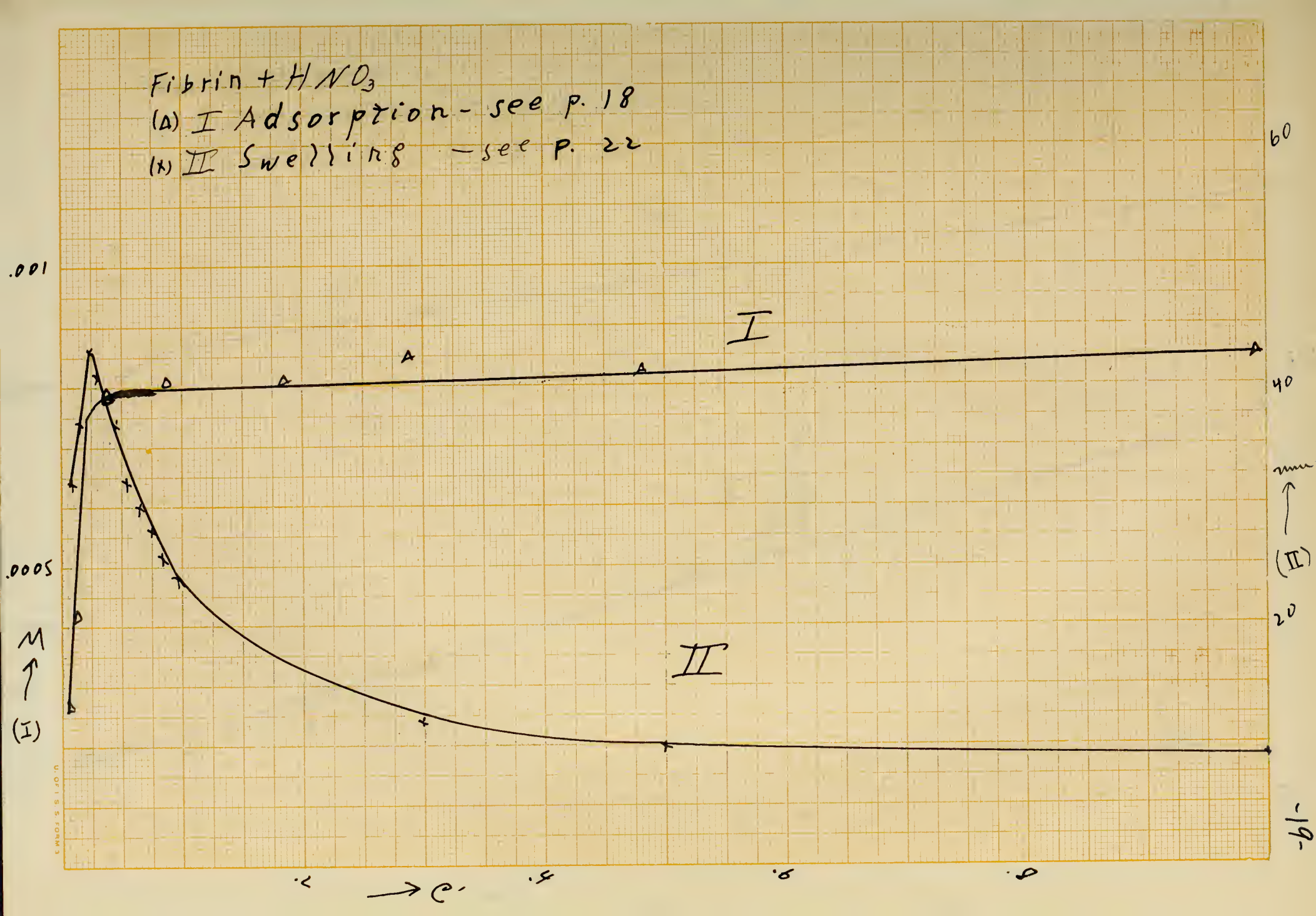
NOTE

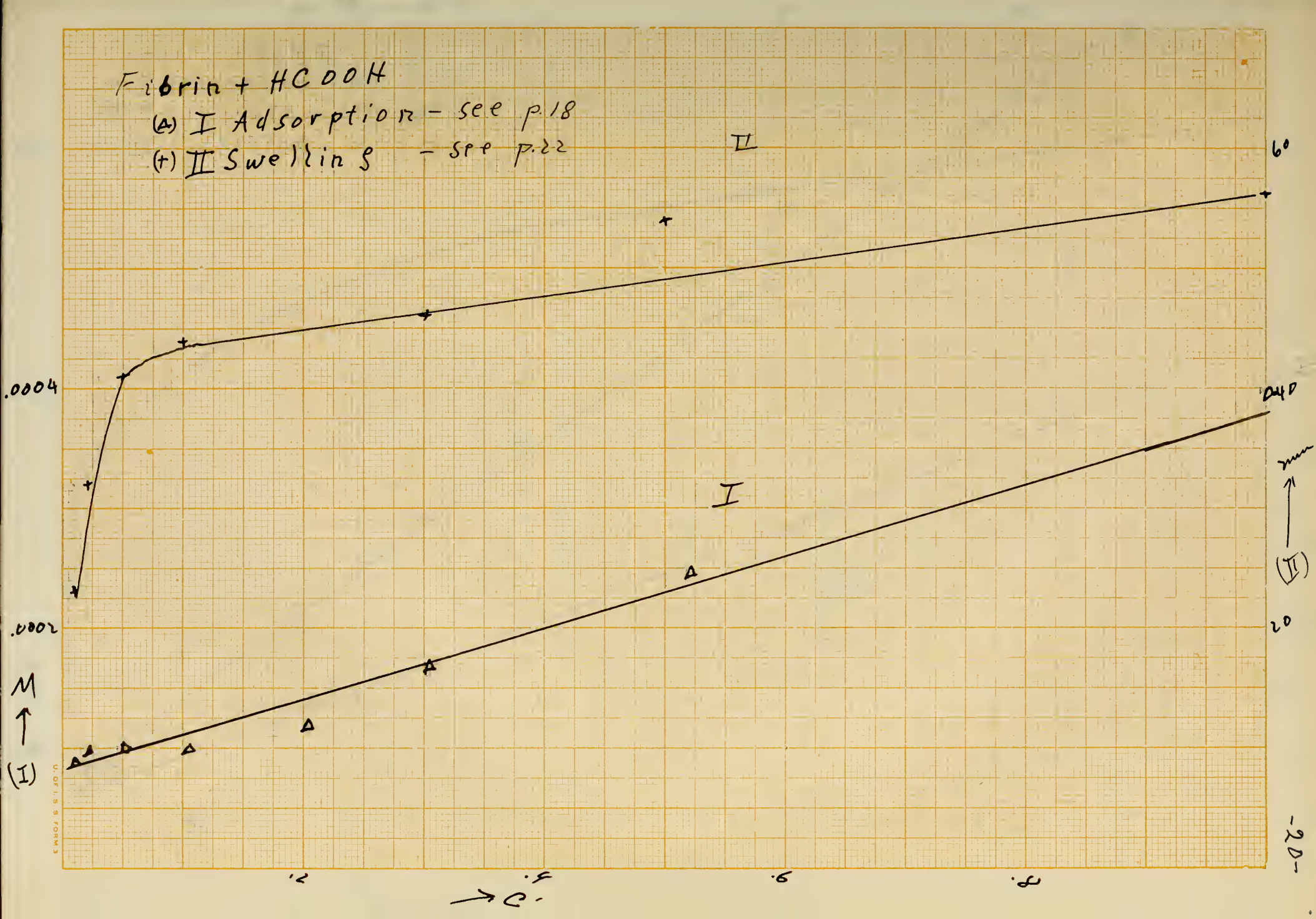
Table VIII-A is valuable only qualitatively as the concentrations run too high for such differences to be determined accurately. It shows that up to 10 N there is no point where the rate of adsorption seems suddenly to change as with mineral acids.

Fibrin + HNO_3

(Δ) I Adsorption - see p. 18

(x) II Swelling — see p. 22




Fibrin + HCOOH
(Δ) I Adsorption - see p. 18
(+) II Swelling - see p. 22
II
I
60
40
20
.0004
.0002
M
(I)
(II)
mm
.2
.4
.6
.8
C.

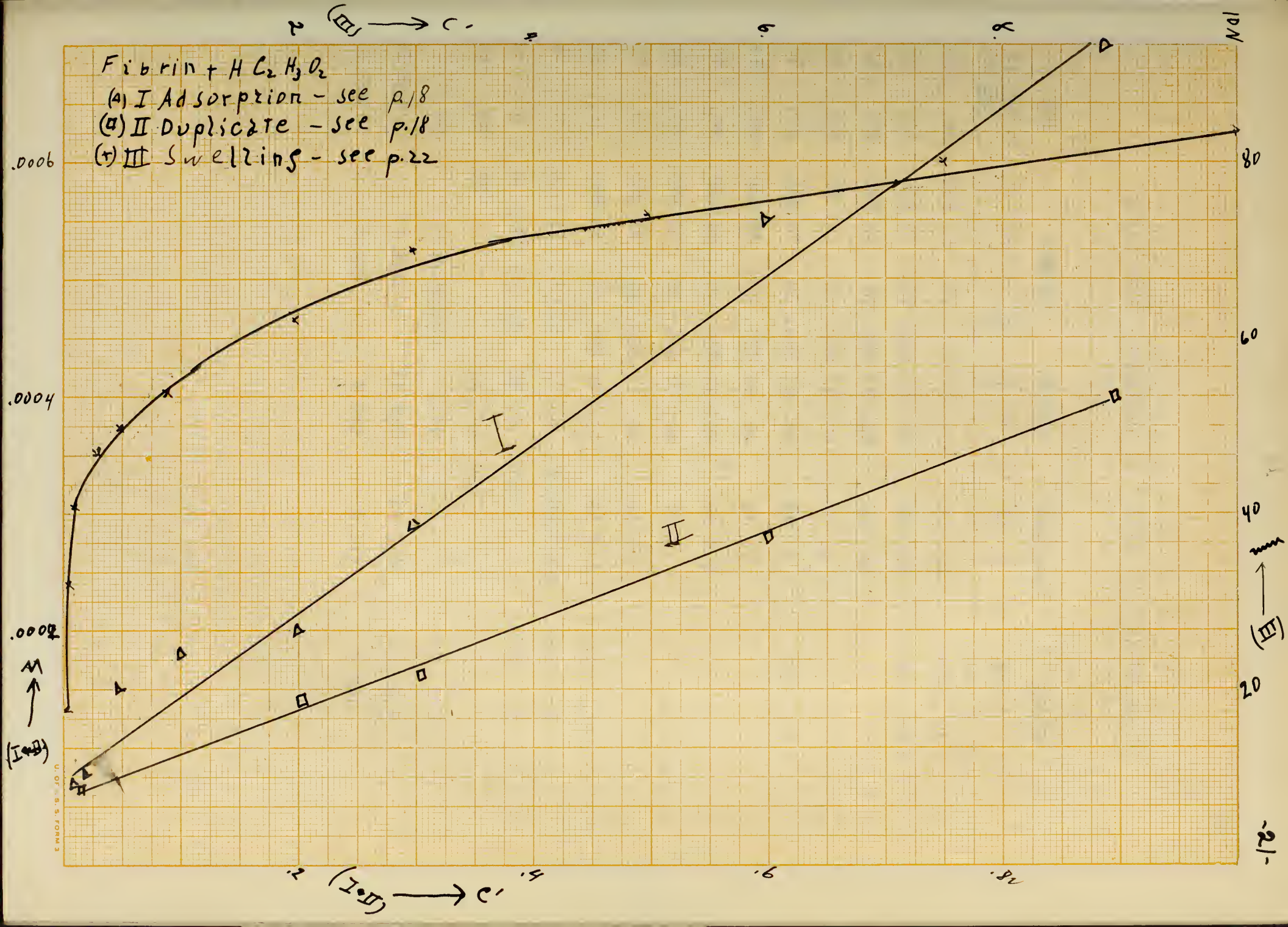

Fibrin + $HC_2H_3O_2$
(A) I Adsorption – see p.18
(a) II Duplicate – see p.18
(τ) III Swelling – see p.22
.0006
.0004
.0002
80
60
40
20
I
II
(III) → C
(I+II) → C'
(I+II)
(III)
mm
.2
.4
.6
.8

TABLE VIII - SWELLING OF FIBRIN IN VARIOUS ACIDS

	HCl			HNO_3			H_2SO_4			$HC_2H_3O_2$			HCOOH		
Height in MM	C	C'	Ht	C	C'	Ht	C	C'	Ht	C	C'	Ht	C	C'
32	.01	.008	32	.01	.008	15	.01		17	.01		23	.01	
40	.02	.016	37	.02	.016	16	.02		32	.05		32	.02	
43	.03	.025	43	.03	.024	18	.03		41	.10				
48	.04	.03	41	.04	.030	19	.04		47	.3				
45	.05	.044	37	.05	.044	19	.05		50	.5		41	.05	
43	.06	.055	32	.06	.055	19	.06		54	.9				
37	.07	.064	30	.07	.067	19	.07		62	2.0				
35	.08	.076	28	.08	.076	17	.08		70	3.				
33	.09	.085	26	.09	.085	17	.09		74	5.				
31	.10	.095	24	.10	.095	17	.10		80	7.5		44	.10	
17	.3	.3	12	.3	.3	17	.3	.3	84	10.00		46	.3	
15	.5	.5	10	.5	.5	15	.5	.5				54	.5	
12	.88	.88				14	.96	.96						
	1.00		9	1.00	1.00		1.00					57	1.00	
11	H_2O		11	H_2O		11	H_2O		11	H_2O		11	H_2O	
5	Dry		5	Dry		5	Dry		5	Dry		5	Dry	

TABLE IX - GELATIN and NaCl*

SAMPLE	C	C'	M
2	1.02	1.040	- .001
2	.5155	.5200	- .00038
2	.3110	.3205	- .00047
2	.202	.2040	- .00017
2	.1040	.1075	- .0001
2	.0522	.0522	
2	.02	.02	
2	.00923	.00923	

TABLE X - FIBRIN and NaCl

SAMPLE	C	C'	M	SWELLINGS (mm)
2	1.020	1.041	- .00105	16
2	.5155	.523	- .000375	13
2	.3110	.3165	- .000275	12.5
2	.2020	.2030	- .00005	11.5
2	.1040	.1045	- .000025	11
2	.0522	.0522		11
2	.02	.02		11
2	.00923	.00923		11

NOTE: *Data was not taken on the swellings since it was so obvious to the eye that the swelling increased with increasing concentration all the way up in both cases.

TABLE XI - FIBRIN and NaCl in .015 HCl V-100cc

SAMPLE	C HCl	C' HCl	n cc HCl adsorbed per grm	C NaCl	C' NaCl	n cc NaCl adsorbed per grm	Adsorbed salt no acid present	Corrected salt adsorption per gram	Swelling in M/10 M
2	.015	.009	3.00	.0125	.009	1.75	0.00	1.75	36
2	.015	.0085	3.25	.0207	.0194	0.65	0.00	0.60	32
2	.015	.0072	3.75	.495	.0485	0.50	0.00	0.50	25
2	.015	.0065	4.25	.1000	.1005	-0.25	-0.25	0.00	20
2	.015	.0038	5.60	.2025	.2056	-1.50	-0.50	-1.00	15
2	.015	.0028	6.10	.3050	.3093	-2.15	-2.75	0.60	12.5
2	.015	.0020	6.50	.5085	.5135	-2.50	-3.75	1.25	11
2	.015	.0013	6.85	1.004	1.0157	-5.85	-10.00	4.15	10

TABLE XI-a

2	.0156	.0008	7.40	2 N					9
2	.07	.0542	7.90	2 N					8
2	1.055	1.039	8.60	2 N					8
							.015 N Acid control		4 8
							Water control		11
							Dry		5

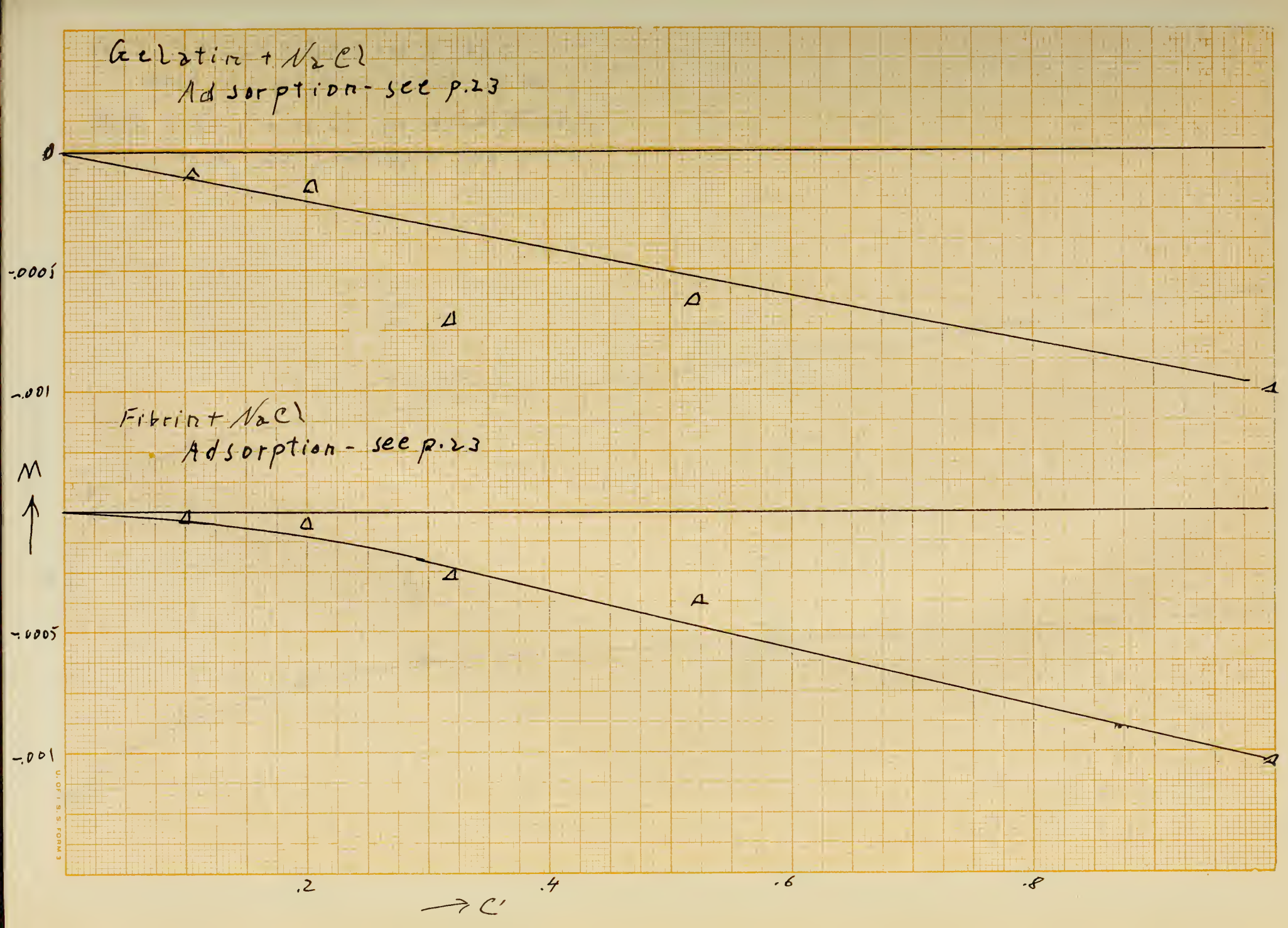
TABLE XII - FIBRIN and ACETIC ACID in N NaCl

V-100 cc

SAMPLE	C Acid	C' Acid	C Salt	M	SWELLING IN M M
2	.018	.0083	N	.00048	11
2	.0343	.0217	N	.00063	10
2	.0867	.0729	N	.00069	10
2	.1730	.1590	N	.00070	10
2	.2175	.2030	N	.00073	9
2	.3187	.3125	N	.00082	9
2	.702	.6835	N	.00092	9
2	1.1510	1.1310	N	.00100	9

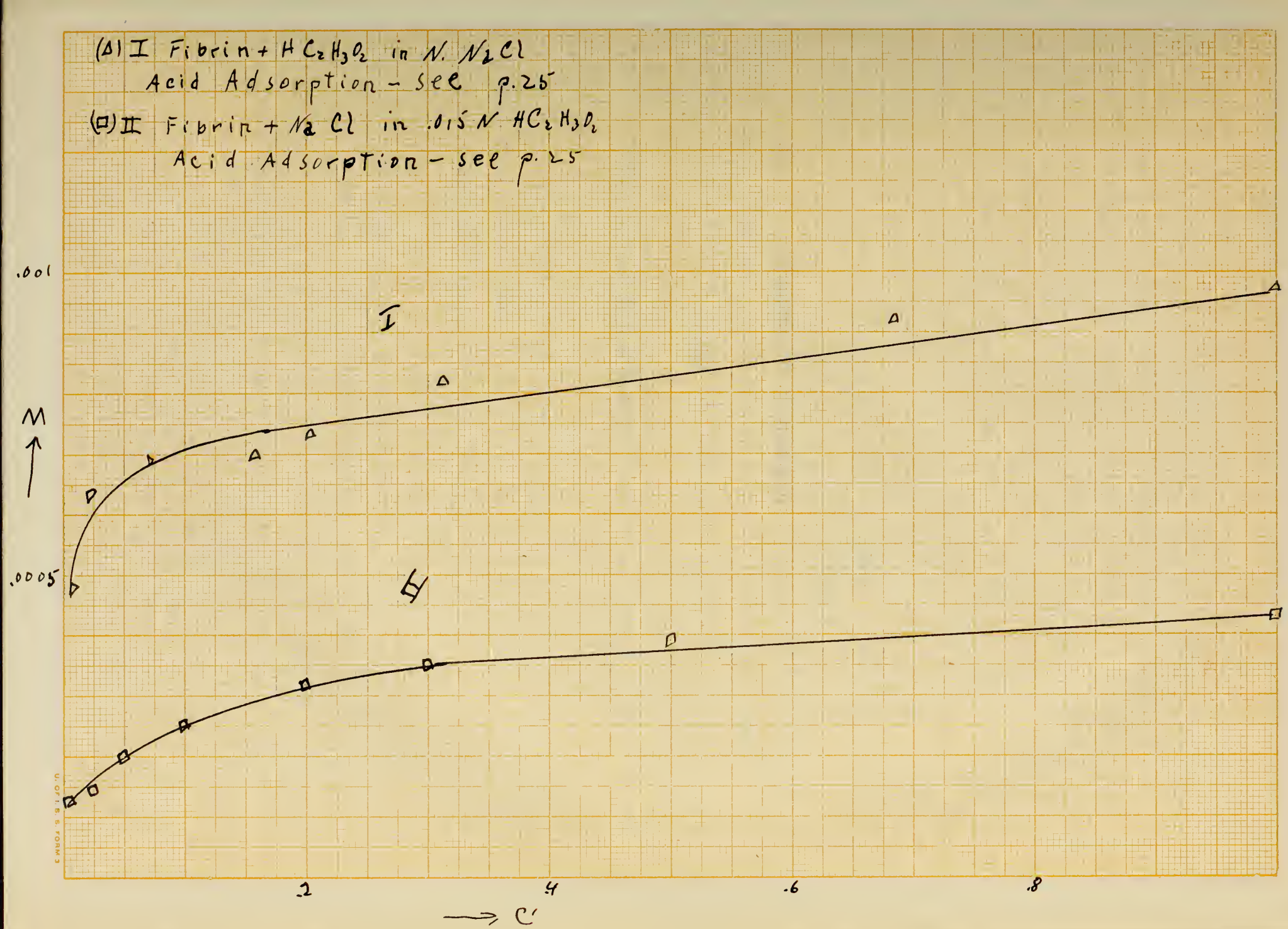
TABLE XIII - FIBRIN and NaCl in .015 N Acetic Acid

2	.0153	.0128	I	.01	.000125	12
2	.0153	.01245	I	.02	.000145	12
2	.0153	.01137	I	.05	.00020	10
2	.0153	.01028	I	.1	.00025	10
2	.0155	.00903	I	.2	.00032	10
2	.0155	.00848	I	.3	.00035	10
2	.0157	.00795	I	.5	.00039	10
2	.0157	.00705	I	N	.00043	10



(Δ) I Fibrin + $HC_2H_3O_2$ in N. $NaCl$
Acid Adsorption – see p. 25

(□) II Fibrin + $NaCl$ in .015 N $HC_2H_3O_2$
Acid Adsorption – see p. 25



IV. DISCUSSION OF RESULTS

AND

V. S U M M A R Y

As may be easily seen there are two general facts in the foregoing data which will not permit of explanation on the basis of a di-acid basic gelatin as put forth by Procter. The first is the well known fact that it does not necessitate a salt with a common ion to knock down the swelling as was mentioned previously; and the second is that gelatin does not behave the same toward weak and strong acids. Inasmuch as Fibrin acted the same as gelatin so far as we could tell, and was easier to work with most of our work was done on it and we shall use it mostly in our discussion.

Perhaps a good deal more depends on the structure of the colloid jelly than one might at first think. Several structures have been assigned. Procter, as was mentioned in an early part of this paper, assumed a molecular solution. Bütschli and Van Bemmelen have observed a porous or sponge like structure and even Procter says this may sometimes be the case.

The theory that gels are systems of two liquid phases is discusses by Hatschek (Bibl. B II) where he shows that this theory must be abandoned unless some structure "both physically possible and showing an entirely different relation between increase of surface and elongation, can be found."

Quinke has suggested that gelatins have a structure similar to that of a sponge. He says they have two phases - the one a phase rich in gelatin and forming the walls of the sponge and the other a liquid phase very weak in gelatin and filling the cells and passages which are formed by the stiff, strong phase.

The work of Mees (Bibl. B XII) on the diffusion of developer in the care of a photographic film seems to support this

view. The small amount of work we have done on that part of the subject this year seems also to indicate the presence of small packets in the structure of the gelatin.

It seems that Procter's data could be equally well explained on the basis of Herzog and Adler's idea of acid adsorption and our data though explainable upon this basis is not so on the asmotic theory. Upon closer observation it may be seen that we are really saying the same thing as Procter regarding the basic phenomenon. He says the HCl adds on to the complex molecule, and then ionizes into Chlorine negative and hydro-gelatin positive. We speak of the same phenomenon and generalize it much more by saying that the hydrogen and hydroxyl ions are selectively adsorbed by protein colloidal gels. This does not preclude a slight adsorption of various other ions to an extent specific for any ion. If a compound were formed analogous to aniline hydrochloride we should not expect to find gelatin swelling in neutral salt solutions. However, we do find slight swellings above the water control even in these salt solutions and this is easily explainable on the adsorption basis by saying that one ion is slightly more adsorbed than another.

The story of the swelling in a solution of a strong acid seems to be about as follows. The acid goes in and the hydrogen ions are adsorbed along the walls of the small pockets. We soon have a layer of positive hydrogen along the wall and of negative anion next and due to electrostatic repulsion the cell is enlarged and water enters.

At very low concentrations the acid adsorbed increases very rapidly with the external equilibrium concentration but at still quite a low concentration the amount seems to approach a

constant quantity and a sharp bend in the curve (page 16) takes place. From here on the amount adsorbed increases only very slightly with relatively large increases in concentration.

The swellings seem to follow a similar curve. They at first increase very markedly above that in pure water and pass a maximum point and then decrease with increasing concentration until they are no greater than the water control. It is significant to note that the point at which the maximum swelling takes place coincides very well with the point where the curve makes its abrupt bend . It seems then to indicate that a strong electrolyte such as HCl will cause a sort of collapse of the cell or a shrinking of the swelling. This is probably due to the fact that the HCl increases the dielectric constant to a large extent, thus weakening the electrostatic repulsion that led to the original enlargement of the cell.

With weak acids such as Acetic or formic acids the curve is a straight line. The swellings also show no maximum at any low concentration but over the range studied increase with the concentration. This difference of behavior may be due to the same type of action exactly and since the effect of neutral salt on both acids is practically the same except in degree we need not assume that the action of the weak acid is different essentially from that of HCl.

It can be explained from the difference in the degree of ionization. From low concentrations of acid the concentration of the hydrogen ion is not so much different. From .0023

N HCl we get the same adsorption as from .009 N $HC_2H_3O_2$ some .00007 mols per gram. As the concentration increases, however the repression of ionization in the weak acid is much greater so that while .03 to .04 N HCl gives an adsorption of .00055 mols per gram, it takes .6 N Acetic acid to do this. The weak electrolyte has also a very much lower dielectric constant so that increasing the concentration even up to 10 N where there would be practically no ionization does not begin to knock down the swelling.

The effect of neutral salts on the swellings seems perfectly analogous to that of the excess of strong acid. When the acid is regulated to a concentration where the maximum swelling would otherwise occur, the addition of increasing amounts of salt cause a decrease in the swellings in the same manner that increases in acid concentrations above this aptimum concentration do. That is the increase of strong electrolyte in the form of a salt increases very markedly the dielectric constant of the resulting solution.

A very striking fact from the data on page 23 is that though acid is adsorbed to a considerable extent, the adsorption of salt from a neutral solution is negative. This may be much better stated by saying that acid is adsorbed to a greater extent than water while salt is adsorbed to a less extent so that from a fairly concentrated solution of neutral salt more water goes into the colloid than salt and the concentration of the salt increases. This effect is perfectly analogous to the effect of salt on the acid adsorption. From a pure HCl solution whose concentration is hundredth normal there will be about .00024 mols of acid adsorbed per gram while if this be in normal NaCl the adsorbed acid will

be about three times as much.

It seems sensible to speak of adsorbed water as well as anything else for several reasons. The volume of the Fibrin in H_2O is over twice its dry volume. This swelling cannot be accounted for by a mere "wetting". Thenthe fact that water is forced in by salt in the absence of acid. From the data on page 23 the no of c.c. of water necessary to account for the change in concentration was calculated and this value compared with the volume of the swelling above that of pure water. The result is Table XIV.

TABLE XIV.

Conc. of salt	.01N	.02N	.05N	.1N	.2N	.3N	.5N	N
H_2O to account for-in C.C.	0	0	0	.05	.1	.55	.75	2.1
Vol. of swelling in excess of pure H_2O - swelling in c. c.	0	0	0	0	.2	.61	.81	2.04

It must be noted that when the heights are so small the relative accuracy in reading them is not great as it is impossible to read between millimeters. We have called anything between the graduations a half a millimeter.

The effect of the presence of an acid is to usurp the place of part of the adsorbed water, since the increase in salt concentration is much less when an acid is present.

The effect of salt in knocking down the swelling of acid is probably very similar to its effect in knocking down the swelling of another salt. Thus while a normal solution of KCl, or of $CaCl_2$ or of NaCl cause a swelling of Fibrin when in pure solution the $CaCl_2$ causing a swelling nearly twice that in pure

water, yet when any two of these salts are mixed the swelling is the same as for pure water. For mixtures of normal KCl and Normal NaCl, and for normal $CaCl_2$ and normal NaCl the height was 11 m.m. the same as for pure water.

With this data in mind it is easy to explain Fischer's statement that the hydrogen ion concentration is not a true measure of the total acidity of a protein if we measure it by titration or indicator methods. We can easily see that, speaking in terms of changes in acid concentration which are physiologically significant, relatively large amounts of acid would at once be adsorbed by proteins without increasing perceptibly the hydrogen ion concentration of the external liquid. Thus a case of acidosis which was very slight so far as one could tell by hydrogen ion concentration might signify a relatively great increase in tissue acidity.

SUMMARY

1. The swelling of protein colloids in acid or alkaline solution is due to selective adsorption of hydrogen or of hydroxyl ions as the case may be.

2. The presence of a neutral salt increases the adsorption of both acid and water.

3. The hydrogen ion concentration of the external liquid as determined by titration or by indicator methods is not a measure of the total acidity of a colloid.

4. The presence of an excess of acid above the amount adsorbed is effective in knocking down the swelling just as is the presence of a neutral salt.

5. Salts of Barium are very destructively effective in tearing colloids to pieces, which fact agrees with the clinical observation that Barium salts are extremely poisonous.

6. The structure of protein colloids is probably sponge-like as suggested for gelatin by Quinke, containing pores or pockets observed by Bütschli and Van Bemmelen.

7. The action of a neutral salt in knocking down the swelling of an acid is not specific for acids or alkalis as one neutral salt will knock down the swelling due to another neutral salt.

8. Common ion action is not an explanation of the action of a salt on an acid swollen protein as any salt is effective. We must look to a property common to all salts.

This work was done in the laboratory of Physical Chemistry of the University of Illinois at the suggestion and under the direction of Professor R. C. Tolman

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